DIVERSITY IN SPAWNING HABITAT ACROSS GREAT LAKES CISCO POPULATIONS

A Thesis

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Master of Science

by

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ABSTRACT

Cisco (*Coregonus artedi*) are a native fish that were historically abundant and a prominent component of Great Lakes ecosystems. However, widespread declines involving the loss of some spawning populations occurred, primarily due to overfishing. This thesis presents observations of diverse spawning habitat preferences among three extant Cisco populations based on the distribution of incubating eggs across depth and substrate gradients. Cisco in Chaumont Bay, Lake Ontario were confirmed to target shallow bedrock shoals for spawning, whereas those in Thunder Bay, Lake Superior and Grand Traverse Bay, Lake Michigan appear to spawn in deeper waters over soft sediment. Evaluations of the diaphragm pump used to sample eggs in the field and the efficacy of using egg size to distinguish Cisco eggs from other species supported this work. The results presented here contribute new information about existing population diversity in support of management decisions targeting Cisco rehabilitation in the Great Lakes.

Matthew Richard Paufve

BIOGRAPHICAL SKETCH

Matt Paufve was born in Rochester, NY in 1992, and from the beginning, he has held a strong connection to the Great Lakes region and a deep curiosity for the natural world. His love of the outdoors and passion for science and ecology led him to pursue a career in biological research. During his undergraduate years, seasonal jobs and research involved studies of wetlands, streams, and lake ecosystems in New York State, and a semester abroad exploring the wildly different ecosystems in Queensland, Australia—where he hopes to return someday. In 2013, he graduated with a Bachelor of Science in Biology from Hobart College. He then worked as a research technician at the USGS Lake Ontario Biological Station, where he was immersed in the field of limnology. He began his Masters studies in the Field of Natural Resources at Cornell University in 2016, where he was also a member of the Cornell Subunit of the American Fisheries Society and co-president of the DNR Graduate Student Assembly. Outside of research and academia, Matt enjoys hiking and cycling, drawing and painting, and caring for his aquariums and orchid collection.

DEDICATION

To my parents, Barb and Rick, for encouraging my curiosity for the natural world, regardless of what critters I brought into the house.

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CHAPTER 1

POPULATION-LEVEL DIVERSITY IN SPAWNING HABITAT USE OF GREAT LAKES CISCO

Abstract

For species that exhibit variation in reproductive strategies, maintaining intraspecific diversity can improve resilience of depleted or recovering populations. Cisco (*Coregonus artedi*) once dominated fish communities in the Great Lakes and spawned on a variety of habitats, but historic declines may have reduced the diversity of populations within the lakes. Rehabilitating extant populations is a management priority, but our understanding of spawning habitat diversity across the basin is insufficient to determine how recovery may be influenced by spawning habitat. We measured the distribution of incubating Cisco eggs in situ across gradients of depth and substrate composition to describe the spawning habitat of three Great Lakes populations. In Chaumont Bay, NY (Lake Ontario), eggs were concentrated on shallow bedrock shoals (1-7 m depth) and not found on deeper silt and sand substrate. In contrast, in Thunder Bay, ON (Lake Superior), Cisco eggs were found on clay mud at deep locations (15-30 m), and none were found on shallower rock substrates. At Elk Rapids, MI (Lake Michigan) eggs were present at sites with silt substrate along a steep depth gradient (11-47 m), and no eggs were found on shallow cobble reefs or sand with scattered rock (1.5-10 m). Our results reflect a broad spawning habitat niche at the species level, but a diversity of spawning habitat preferences at the population level. Efforts to maintain this diversity and support Cisco rehabilitation in the Great Lakes will benefit from further evaluation of spawning strategies exhibited by extant populations.

Introduction

Spawning habitat use is an important component of fish life history and can impact recruitment success and population abundance if available habitat is degraded or scarce (Hayes et al. 1996, Rosenfeld and Hatfield 2006). Cisco (*Coregonus artedi*) are a pelagic, cold-water, planktivorous

fish native to the Laurentian Great Lakes that migrate from pelagic zones to form discrete spawning aggregations in fall, often in embayments or nearshore areas over specific habitat types (Koelz 1927). Spawning occurs in late November to early December, when eggs are broadcast into the water column and settle to the substrate where they overwinter until hatching in early spring (Dryer and Beil 1964). Thus, the habitat selected for spawning directly determines the conditions faced by incubating eggs, and could be a critical aspect of their early life history (Freeberg et al. 1990, Madenjian et al. 2011).

Historically abundant, Cisco held a prominent role in native ecosystems as prey for Lake Trout (*Salvelinus namaycush*) and Atlantic Salmon (*Salmo salar*) and supported commercial fisheries in all five lakes (Baldwin and Saalfeld 1962, Dryer et al. 1965, Madenjian et al. 2008). However, fisheries exploitation, interactions with nonnative species, and habitat degradation contributed to widespread population collapses in the mid-1900s reflected in the current existence of local extirpations and remnant populations (Brown et al. 1999, Christie 1974, Madenjian et al. 2008). Some population recovery has occurred in Lake Superior—where populations are large enough to support small commercial fisheries, but are still considerably less abundant relative to historic levels (Stockwell et al. 2009). There is limited evidence of population growth in lakes Ontario, Michigan, and Huron, and Cisco remain extirpated from Lake Erie (Great Lakes Fishery Commission 2018, US Geological Survey Great Lakes Science Center 2017). Due to their past significance in the lakes and their potential to increase the available diversity of prey fish, management objectives prioritize the rehabilitation of extant Cisco populations in the Great Lakes (e.g. Great Lakes Fishery Commission 2017, Stewart et al. 2017).

Evaluating possible management strategies, however, depends on a sufficient foundational knowledge of Cisco spawning behaviors and early life history. At the species level, Cisco are described as spawning habitat generalists (Goodyear et al. 1982). Indeed, a review of observations of spawning habitat use in the Great Lakes from the literature (Table 1.1) illustrates the broad spawning habitat niche of the species; in shallow and deep offshore waters, over essentially all substrate types, and even in rivers. However, there is evidence of natal philopatry at the population level and abundant

reports of distinct spawning strategies related to specific habitat use (Bronte et al. 1996, Todd and Smith 1992). Speciation in other Coregonine species has been attributed to spatial and temporal reproductive isolation resulting from plasticity and diversity in spawning strategies (Fenderson 2005, Vonlanthen et al. 2012). This plasticity and capacity for local adaptation may also explain the expansive historical range of Cisco across heterogeneous habitats in the Great Lakes (Turgeon et al. 1999). However, patterns of spawning habitat use in Cisco are not well characterized at the population level, especially for extant populations (Bronte et al. 2017).

To address knowledge gaps in Cisco life history and inform management of existing Cisco populations in the Great Lakes, we documented patterns in population-level spawning habitat use across a broad spatial scale at three known spawning areas: Chaumont Bay, NY (Lake Ontario), Elk Rapids, MI (Lake Michigan), and Thunder Bay, ON (Lake Superior). During the winter incubation period, we characterized fine-scale habitat and sampled the substrate for Cisco eggs *in situ* across gradients of depth and substrate in each area. By describing the distribution of eggs among habitat types, we sought to make inferences about the spawning habitat preferences of these populations.

Table 1.1. Summary of published observations of spawning habitat use of Cisco in the Great Lakes from primary literature and reviews. “Shallow” and “deep” were defined approximately according to a 10 m threshold.

Lake	Time of observations	Reference	Reported spawning habitat characteristics
Ontario	Late-1800s to early-1900s	1	Shallow, over mud, sand, gravel and cobble reefs, bedrock shoals.
			Deep water in western end of the lake, miles offshore.
			Evidence of spawning runs in the Niagara River.
	2010s	2	Shallow bedrock shoals.
Michigan	Late 1800s to mid-1900s	1	Mostly shallow, some deeper, on all substrates (mud, clay, sand, gravel, rock).
			Evidence of spawning in deep water over mud.
	2000s	10	Shallow nearshore.
Superior	Late-1800s to 1970s	1	Shallow along shorelines, all substrates (mud, clay, sand, rock, shoals).
			Shallow, targeting reefs and rocky substrates ("reef" herring).
			Offshore, pelagic spawning over mud ("deepwater" or "mud" herring).
		5	Evidence of spawning in the French River.
	1960s to 2000s	6	Shallow, over rocky reefs and in bays.
		6, 7	In deep water, possibly pelagic spawning.
	2000s	8, 9	Deep, offshore.
Huron	Late-1800s to 1970s	1	Along entire shoreline, various substrates.
			Shallow nearshore, on various substrates in St. Marys River.
	1990s	3, 4	Shallow nearshore, on various substrates in St. Marys River.
	2000s	10	Shallow nearshore.
Erie	Late 1800s to mid-1900s	1	Shallow nearshore and "in open water over all bottom types."
			Rapids in the Detroit and Maumee Rivers.
			Offshore, deep relative to maximum lake depth.

*^†‡	1 Goodyear, C. D., et al. 1982	* Observations of egg deposition or incubating eggs
*	2 George, E. G., et al. 2017	^ Direct observations of spawning adults
^	3 Savino, J. F., et al. 1994	† Observations of adults assumed to be spawning
*†	4 Fielder, D. G. 2000	‡ Some observations not confirmed to be *C. artedi*
†	5 Selgeby, J. H. 1982	
^	6 Ebener, M. P., et al. 2008	
*	7 Dryer, W. R., and Beil, J. 1964	
^	8 Yule, D. L., et al. 2006	
†	9 Stockwell, J., et al. 2009	
†	10 Bronte, C. R., et al. 2017	

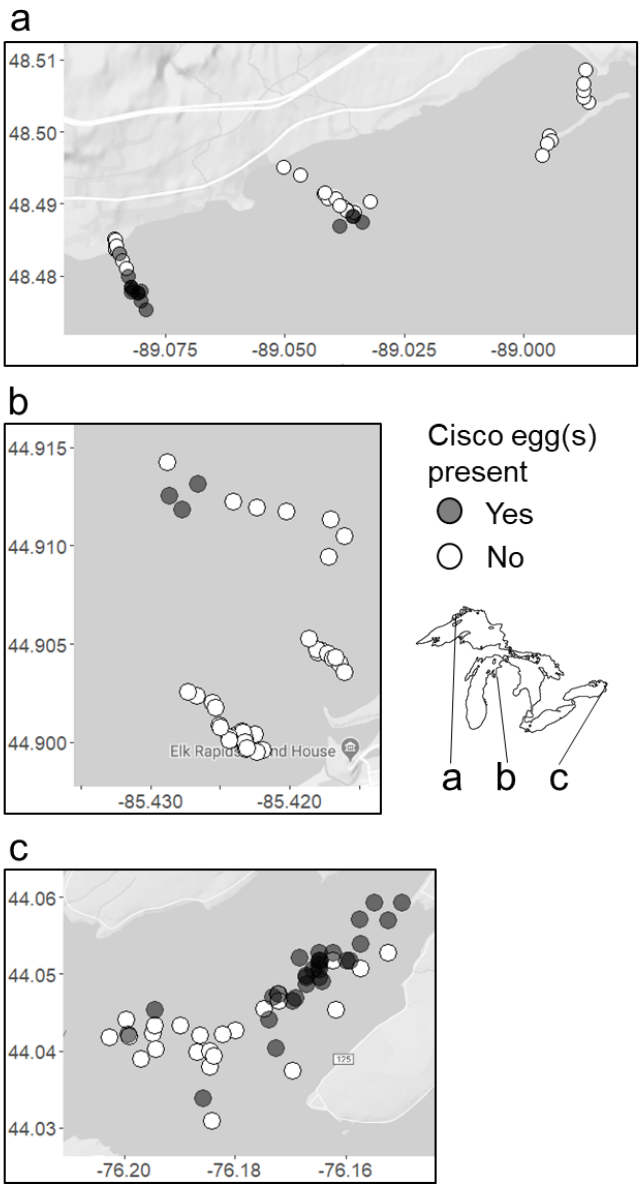


Figure 1.1. Location of sampling sites and Cisco egg presence within our three study areas: (a) Thunder Bay, Lake Superior, (b) Elk Rapids, Lake Michigan, and (c) Chaumont Bay, Lake Ontario.

Methods

Study areas

We selected three study areas with established Cisco populations, some evidence of spawning, and that offer a range of depths and substrate types which reflect the diversity of nearshore habitat types potentially used by Cisco (Figure 1.1). Chaumont Bay, NY (Lake Ontario) is the shallowest of the three areas (maximum depth ~ 15 m), and is characterized by shallow, smooth ridges of bedrock shoals surrounded by deeper areas of sand and silt. George et al. (2017) confirmed spawning on this shoal habitat, and we sought to confirm whether Cisco target the shoals or may spawn elsewhere in the bay. Elk Rapids, MI (Lake Michigan) is located along the eastern coast of the east arm of Grand Traverse Bay, and is characterized by nearshore expanses of clean sand and clay marl interspersed with long, narrow cobble reefs, with a steep drop-off defining the edge of the nearshore area (Barton et al. 2011). While ripe adults have been captured near Elk Rapids and a remnant population is noted in the east arm Grand Traverse Bay (Stockwell et al. 2009), it is unclear if the reefs or other areas are being used for spawning. Our third study area is located along the northwest shoreline of Thunder Bay, ON (Lake Superior), where historical records from commercial fisheries and recent surveys of spawning aggregations suggest that Cisco spawn in relatively deep waters—presumably over mud and silt substrate, although rocky substrate is also present in the bay (Goodyear et al. 1982, Yule et al. 2009).

Egg sampling and habitat characterization

We sampled eggs from the substrate using a gasoline-powered diaphragm pump, as this approach allowed us to sample incubating eggs *in situ* and facilitated sampling through ice cover when present (Paufve et al. 2019). Ribbed pool hose connected the pump to a conical steel intake (22.8 cm diameter opening) deployed through an ice auger hole or from a boat if open water was present. The intake was lowered to the bottom and sat upright on the substrate during pumping. We timed sampling to last for two minutes, although sampling on clay and mud substrates was often terminated after 30 seconds to prevent the intake from becoming stuck. Outflow was filtered through a mesh bag and any

collected materials were rinsed into plastic bags and kept cold until inspected for eggs, which were removed and preserved in 95% ethanol. In Chaumont Bay, we supplemented the pump samples with samples collected using egg mats—steel frames (approximately 40 x 20 cm) covered on each side with a layer of hog hair furnace filter secured with binder clips (e.g. Roseman et al. 2011). Mats were deployed individually with a small buoy and anchor and rested on the substrate during spawning. Eggs settling out of the water column onto the mats became trapped in the fibers. Upon retrieval, mats were unclipped from the frames and stored in plastic bags until eggs were removed and preserved in 95% ethanol the same day. Egg deposition rates were estimated for egg mat samples, but we do not report these values as we only considered them to be interpretable as coarse indicators of relative spawning activity. We interpreted all data from both sampling methods as observations of egg presence to accommodate pooling results and to account for known differences in the effectiveness of the pump sampler on different substrate types (Paufve et al. 2019).

To characterize benthic habitats, we recorded benthic imagery at each egg sampling site using a SeaDrop HD underwater camera (SeaViewer, Inc., Tampa, FL), and measured dissolved oxygen content at 1-5 cm above the substrate using either a YSI ProODO or YSI 550a handheld probe (YSI, Inc., Yellow Springs, Ohio). Distinct substrate habitat types were defined for each study area by grouping observations of similar substrate particle size composition, determined from benthic imagery. In Chaumont Bay, sites were categorized as mussel-encrusted bedrock shoals (1-7 m depth), sand and scattered gravel areas around the shoal perimeters (3.6-8.5 m), and a mixture of sand and silt (5.7-10 m). Habitat types at Elk Rapids consisted of patchy reefs of clean, rounded cobble (1.8-2.4 m), interspersed among expanses of compacted sand and exposed clay marl with scattered patches of embedded gravel and cobble (1.5-8.5 m), clean sand flats extending from the shallow areas of the first two habitats to the edge of a steep depth gradient (4.5-10 m), and soft silt substrate with patches of *Dreissena* mussels (11-47 m) extending beyond the sand flats. In Thunder Bay, habitat types consisted of piled broken rocks in shallow zones (2.1-9.3 m), compacted sand with patches of scattered rock of

various sizes (1.6-15.1 m), a mixture of silt and sand (2-24 m), and softer clay mud in deeper areas (15-30 m).

Table 1.2. Sampling effort per study area by sampling method and time.

Location	Year	Month	Method	No. samples
Chaumont Bay, NY	2017	Dec.	Mats	30
	2018	Dec.	Mats	14
	2018	Jan.	Pump	5
	2018	Feb.	Pump	4
Elk Rapids, MI	2016	Dec.	Pump	16
	2018	Mar.	Pump	24
Thunder Bay, ON	2017	Apr.	Pump	27
	2018	Feb.	Pump	20

Pump sampling and concurrent habitat characterization were conducted over three winters between 2016 and 2019. Egg mats were deployed in Chaumont Bay during the spawning season in 2017 and 2018 (Table 1.2). Most pump sampling was completed in January or later with the intention of observing egg condition as an indicator of survival (e.g. George et al. 2017). Specific timing varied due to the logistics of targeting multiple study areas, periods of inclement weather, and ice conditions that prevented working from a boat or on the ice. In addition, sampling needed to take place after the conclusion of spawning and prior to the initiation of hatching to ensure that egg deposition was complete and that our samples would reflect the distribution of incubating eggs. In Elk Rapids, some sampling occurred in late December of 2016, after we assumed spawning had concluded (Dryer and Beil 1964). In Thunder Bay, some sampling occurred in late April, 2017, approaching the expected hatching period. We collected several four-minute neuston net tows from a boat and found no fish larvae, therefore, we concluded that hatching had not yet begun.

Egg species identification

Cisco and closely-related Lake Whitefish may use the same spawning grounds, and their eggs are similar in appearance, necessitating species confirmation of eggs in our samples (Behmer et al. 1980, Pritchard 1931). Two other species could potentially overlap in spawning location and incubation period; Lake Trout eggs are larger and easily distinguished from Cisco and Lake Whitefish eggs, and bloater (*Coregonus hoyi*) spawn in relatively deep areas later in winter and were unlikely to be spawning at our study areas. Thus, we confirmed the species identity of eggs as Cisco or Lake Whitefish using DNA barcoding of the mitochondrial cytochrome c oxidase I (COI) gene following the protocol used by George et al. (2017). Large sample sizes collected with egg mats in Chaumont Bay were subsampled to select eggs for genetic analysis. DNA was extracted from eggs using the DNEasy Blood & Tissue Kit (Qiagen, Valencia CA), PCR was used to amplify extracted DNA with primers VR1_t1 and VF2_t1 (Ivanova et al. 2007), and the resulting COI gene copies were fragmented using the restriction enzyme Eco109I (George et al. 2017). Restriction fragment length polymorphisms of the COI gene were visualized using gel electrophoresis, and banding patterns for individual eggs were compared to known samples run in the same gels. DNA extraction was not successful for all Elk Rapids eggs (n = 6), and one egg from Thunder Bay. We assumed that these were likely Cisco eggs based on visible differences in size (Paufve 2019), and further supported by gillnet captures of ripe adult Cisco during the preceding spawning season in the same location (J. Smith, pers. comm., 2017). Eggs genetically identified as Lake Whitefish (n = 9 across all samples) were removed prior to data analysis.

Data analysis

To assess whether habitat preferences exist in each population, we used Jacob's Electivity Index (*D*) to compare the proportional distribution of egg detections with the proportional distribution of samples across substrates and depths (Jacobs 1974). *D* was calculated separately for substrate habitat types and for relative depth areas, as substrate type and depth were correlated. We categorized sites as "shallow", "mid-depth", and "deep" habitats using the 5 and 10 m depth contours. As *D* is

bounded [-1, 1], positive values were interpreted as habitat preference and negative values suggested avoidance. To test the null hypothesis that Cisco do not select certain habitats, we implemented permutation tests to estimate *D* for 10,000 iterations of randomly redistributing positive detections across the habitat types or depth ranges sampled.

Results

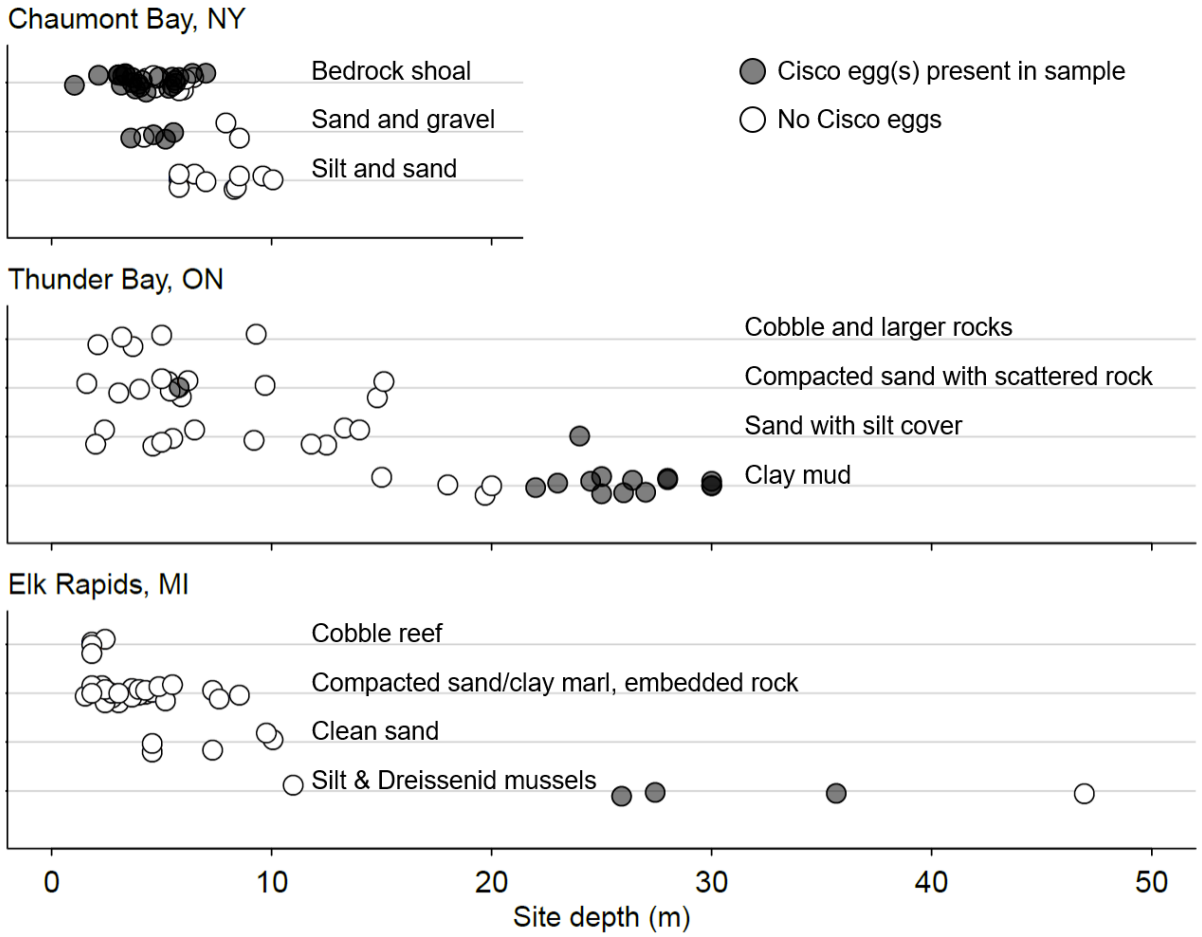
Cisco eggs were present at sites that ranged in depth from 1 to 47 m, and on substrates that included clay mud, silt with *Dreissena* mussels, silt covered sand, sand with gravel, compacted sand with embedded rocks, and bedrock shoals (Table 1.3, Figure 1.2). Most egg detections occurred at shallow, rocky sites and deep, soft substrate sites, with relatively few at mid-depths (Table 1.3). Across all sites, dissolved oxygen concentrations immediately above the substrate were high, and near or above saturation. Mean oxygen measurements were 11.62 (SD = 1.32; n = 23) mgL^{-1} in Chaumont Bay, 13.32 (SD = 1.16; n = 39) mgL^{-1} at Elk Rapids, and 13.07 (SD = 1.06; n = 41) mgL^{-1} in Thunder Bay.

Table 1.3. Egg presence and number of samples by study area, substrate habitat type, and depth category. Dashes indicate substrate and depth combinations not present in our data. Jacob's electivity index values (*D*, Jacobs 1974) were calculated separately for habitat types and depth categories. Permutation tests (10000 iterations) were used to derive *p*-values. *D* is bounded [-1, 1], and values farther from zero suggest preference (positive, bolded values) or avoidance (negative).

Location	Substrate habitat	Shallow (≤ 5 m)	Mid-depth (5-10 m)	Deep (> 10 m)	*D (p)*
Chaumont Bay, NY	Bedrock shoal	17/21	8/14	-	**0.55 (0.0014)**
	Sand and gravel	2/3	2/4	-	**0.28 (0.61)**
	Silt and sand	-	0/11	-	-1 (0.0001)
Elk Rapids, MI	Cobble reef	0/4	-	-	-1 (0.71)
	Compacted sand and clay marl, embedded gravel and cobble	0/21	0/5	-	-1 (0.038)
	Clean sand	0/2	0/2	0/1	-1 (0.66)
	Silt with dreissenid mussels	-	-	3/5	**0.29 (0.0012)**
Thunder Bay, ON	Cobble and larger rocks	0/4	0/1	-	-1 (0.14)
	Compacted sand with scattered rocks of various sizes	0/4	1/7	0/2	-0.65 (0.024)
	Sand with silt cover	0/4	0/3	1/5	-0.62 (0.041)
	Clay mud	-	-	13/17	**0.47 (< 0.0005)**

	D (p)		
Chaumont Bay, NY	**0.39 (0.001)**	-0.39 (0.001)	-
Elk Rapids, MI	-1 (0.029)	-1 (0.55)	**1 (0.002)**
Thunder Bay, ON	-1 (0.0035)	-0.62 (0.061)	**0.86 (< 0.0005)**

Figure 1.2. Visualization of Cisco egg presence across the substrate habitat types (vertical axis, labeled gray lines, points jittered to reduce overlap) and depths (horizontal axis) of sampling sites across three study areas. Circles filled with transparent black indicate at least one Cisco egg was present in the sample, and white-filled circles indicate an absence of Cisco eggs in the sample. Top panel (Chaumont Bay) is truncated as the maximum depth sampled was approximately 10 m.



Within each study area, we observed specific habitat preferences at the population level that generally targeted shallow, rocky substrate or deeper, fine sediment. In Chaumont Bay, Jacob's electivity index values (Table 1.3) reflected preference for bedrock shoal substrate (D = 0.55; p = 0.0001) and a weaker preference for adjacent sand and gravel substrate (0.28; $p > 0.05$). This was consistent with relative egg deposition rates estimated from egg mat samples, which were generally highest at the shallowest parts of the shoals and decreased outward from the shoals. Shallow depths associated with the bedrock shoals were also preferred (0.39; $p = 0.001$; ≤ 5 m) over deeper areas

where no eggs were found. At Elk Rapids, an absence of eggs in all nearshore samples and presence in samples collected along a steep offshore depth gradient indicated a preference for silt substrate (0.29; $p = 0.0012$) and deep areas (1; $p = 0.002$; > 10 m). Within our Thunder Bay samples, we observed a preference for deep (0.86; < 0.0005; > 10 m), soft substrate (0.47; $p < 0.0005$), though two eggs were collected from a single mid-depth (5-10 m) site with scattered rock and compacted sand.

Discussion

Our results provide direct *in situ* characterization of Cisco spawning habitat use for three spatially distinct populations in the Great Lakes. Cisco in Chaumont Bay target the shallow bedrock shoals over deeper, fine substrates, as proposed by George et al. (2017). Eggs were also present at some sites with sand and gravel substrate surrounding the shoals, likely representing spillover from targeted spawning on the shoals. This conclusion is supported by estimates of egg deposition rates from our egg mat samples, which were highest at the shallowest parts of the shoals and tapered off into the surrounding areas. In contrast, Cisco in Thunder Bay and Elk Rapids appear to target deeper areas with soft sediment—predominantly clay in Thunder Bay, and accumulated silt with fine organic debris at our sites in Elk Rapids. The differences we observed in spawning habitat preferences reflect plasticity in spawning habitat use at the species level, though we caution drawing specific inferences beyond the populations and substrate-depth combinations that we studied.

Across the Great Lakes, some diversity in Cisco spawning strategies appears to be maintained, but comparing historic descriptions with our observations suggests that the current diversity within lakes may be lacking. For example, in Grand Traverse Bay, commercial fishers noted that Cisco spawned in shallow nearshore areas over sand substrate (Koelz 1927), a spawning strategy not evident in our results or noted in recent times. In Lake Ontario, Goodyear et al. (1982) reported evidence of deep-spawning Cisco in the west end of the lake in the early 1900s, a strategy not thought to be present in the lake today. Cisco also once spawned over mud in shallow water (Stone 1938) in Irondequoit Bay, Lake Ontario, and evidence suggests this is has no longer been the case for many

decades (George et al. 2018, B. Weidel pers. comm. 2019). There is insufficient evidence, however, to determine if these strategies were lost, or if they might exist but remain undetected.

Various mechanisms are thought to have contributed to a past reduction of diversity in spawning strategies within the lakes. During the height of Cisco fisheries in the early to mid-1900s, sequential exploitation likely resulted in the loss of discrete spawning populations (Selgeby 1982) and possibly the loss of some specific spawning strategies. Larval predation by nonnative Alewife and Rainbow Smelt and spatial variation in the abundance of these species may have also had an influence on which populations and spawning strategies persisted. Some have suggested spawning habitat degradation as another factor contributing to local declines, and as a possible influence on population abundance today (Madenjian et al. 2011).

Spawning habitat limitation has been posed as a possible barrier constraining population expansion, however, insufficient information exists to evaluate this hypothesis (Fitzsimons and O'Gorman 2006). Currently available information about Cisco spawning habitat use largely consists of unsubstantiated, historical reports from commercial fisheries or other anecdotal evidence. Thus, our current understanding of habitat preference lacks quantitative and spatially comprehensive context that could inform management decisions regarding habitat restoration and other actions (Bronte et al. 2017). The information we present in this study represents progress in filling some of the existing knowledge gaps. However, we did not specifically evaluate habitat selection by comparing habitat use with the extent of available habitat, which would have required a different sampling approach to define the total habitat space available to a spawning population.

We stress that systematic, comprehensive sampling designs are important when assessing selection processes and habitat preference. Our sampling effort was not evenly distributed across all available habitat types, partly reflecting the actual extent of each type and partly due to field conditions and challenges in collecting samples. For example, few samples were collected at mid depths on sand at Elk Rapids relative to the large extent of this habitat zone. All sampling at this study area was conducted from a boat, and we had difficulty anchoring on bare sand in windy conditions.

Considering the challenges in collecting these data, it is important that research questions align with appropriate sampling approaches for related studies in the future.

Operation of the diaphragm pump that we used as our primary sampling method may have influenced results if factors like substrate complexity or depth unevenly influence the susceptibility of eggs to the sampling gear. Our pump sampler was evaluated on different substrates and found to be less effective on cobble reefs (Paufve et al. 2019). In response, we limited the sampling effort on cobble reefs at Elk Rapids, resulting in weak power to conclude a lack of Cisco spawning on this habitat type. Further study of the cobble reef at Elk Rapids would require alternative sampling methods such as those described in Barton et al. (Barton et al. 2011). Overall, the pump sampler allowed us to characterize spawning habitat use in the three populations, although the egg mat surveys conducted in Chaumont Bay proved important for supplementing presence data with relative measures of spawning intensity. Ideal sampling gear for this project would allow deployment broadly across habitat gradients, exhibit consistent sampling behavior, provide quantitative data, and be reliable and easy to use in winter field conditions.

Factors other than substrate and depth could be important determinants of habitat suitability; for instance, Cisco eggs require dissolved oxygen content to be above a certain threshold to survive (Brooke and Colby, 1980). We encountered oxygen concentrations that were consistently high across depth and substrate types, and therefore not likely to influence successful egg development. Decreased oxygen concentrations could be associated with other habitat characteristics, such as nutrient inputs and limited rates of exchange. In addition, other factors, such as energy regimes (i.e. wave energy and current flow) or egg predation may also impact spawning and incubation success (Bouwes and Luecke 1997, Ventling-Schwank and Livingstone 1994). Patterns in these habitat components may explain some benefits and costs of spawning habitat preferences, although diversity of habitat use can also be explained by other large-scale processes.

If preferences for specific habitat components, like depth and substrate, are conserved across populations, strategically transplanting individuals from certain populations could increase diversity

within a lake and allow the lake-wide assemblage to exploit additional spawning habitat. Shields and Underhill (1993) found that spawning timing may be constrained in Cisco transplanted between lakes, but that size differences were not. It remains unclear if spawning habitat preferences are also constrained. Transplantation is one possible approach to supporting rehabilitation of Cisco, but it is based on the assumption that extant populations may represent only a subset of historical or Great Lakes-wide diversity (Bronte et al. 2017). While much work remains to be done to understand the existing diversity of spawning strategies, our study contributes quantification of spawning strategies exhibited by three remnant populations and provides new information specific to these populations than can guide future studies.

CHAPTER 2

ASSESSING THE SPAWNING ECOLOGY OF FISH IN SITU USING A BENTHIC PUMP SAMPLER

Abstract

In situ observations of incubating fish eggs can identify spawning sites and spawning habitat preferences, informing the ecology of fishes with benthic eggs. Suction pumps have been used to sample benthic-incubating, non-adhesive fish eggs, yet their sampling efficiency is not well known. Imperfect or systematically variable egg detection could bias resulting ecological inference if left unaddressed. Here we present results from replicate field trials and examine the effects of varying substrate type, intake design, sampling effort, and egg density on the ability of a gasoline-powered diaphragm pump, rated for 66 gal/min (approx. 250 L/min), to detect egg presence and estimate relative or absolute abundance. A wider box-shaped intake was effective at detecting the presence of eggs on fine, silty substrates, but had limited effectiveness on larger-grained substrates. A narrower cone-shaped intake consistently detected the presence of eggs on silt, gravel, and shallow cobble, and demonstrated potential to measure relative and absolute egg abundance. Neither intake design was able to collect eggs from deep interstitial spaces (e.g. several layers of cobble), indicating that there are limitations to the types of substrates on which benthic sampling pumps can operate. Both intake designs often collected eggs from outside the edge of the intake opening. Most eggs were collected within a two minute period, but increasing pumping time to four minutes produced better egg detection outcomes. Our results suggest that stationary benthic sampling pumps are viable tools for directly sampling incubating eggs on most substrates, but have imperfect and varying sampling efficiency resulting from intake design and substrate type.

Introduction

Benthic egg incubation is a reproductive strategy of many freshwater, estuarine, and marine fishes (Balon 1975). For these species, substrate conditions may influence the survival of incubating eggs and ultimately impact recruitment success (e.g. Freeberg et al. 1990). Thus, in situ observations of egg deposition and incubation directly from benthic habitats can provide information useful for the management of these populations and can guide habitat restoration or conservation efforts.

Previous studies have inferred spawning habitat preference and survival of non-adhesive benthic eggs using passive sampling gear such as egg traps. These, however, provide a measure of survival on modified substrate, as the traps either act as artificial substrate (e.g. egg bags or mats; Horns et al. 1989, Roseman et al. 2011) or require alterations to the substrate for deployment (e.g. buried mesh bags; Barton et al. 2011, Perkins and Krueger 1984). Alternatively, active sampling gears such as water pumps have been used for in situ collection of incubating eggs on natural substrates (e.g. Bouwes and Luecke 1997, Collins et al. 2000).

These pumps typically utilize a diaphragm or centrifugal design to create suction through an intake to lift available eggs upward from on or within the substrate. In previous studies, pumps have been deployed with intakes mounted to benthic sleds (Manz 1964) or remotely operated vehicles (Marsden 1997), and others operated as stationary samplers from a boat (Viljanen 1980), by divers (Flath and Dorr III 1984, Stauffer 1981), or through ice (Bouwes and Luecke 1997, George et al. 2017). Stationary pump samplers, designed for point sampling rather than being dragged over a larger area, enable targeted sampling of habitats and could potentially provide data suitable for quantitative analysis. When specifically used to collect fish eggs, diaphragm-type pumps may be preferred as they are assumed to be less likely to damage eggs (Flath and Dorr III 1984).

Because these pumps work by mechanical action, with direct contact between the intake and sampling surface, their efficacy at collecting eggs may vary by the type of substrate being sampled. Imperfect or systematically variable egg detection could bias ecological inference if left unaddressed (Mackenzie et al. 2002, Royle and Dorazio 2008). However, we have not found detection efficiency

estimates in the literature for the application of a diaphragm pump for sampling benthic, non-adhesive eggs in situ. To address this, we conducted a controlled field experiment to assess the effectiveness of a stationary, surface-operated diaphragm pump sampler at collecting seeded eggs across a range of substrate types and egg densities.

Eggs deposited on substrates with complex or deep interstitial space, such as cobble reefs, could be less susceptible to a sampling pump if they have settled into the interstices. Thus, we selected a field site for this experiment that enabled us to test the pump sampler on four distinct habitat types that represent a gradient of interstitial space and reflect common benthic habitat types in freshwater systems: silt, a single layer of gravel, a single layer of cobble, and cobble three layers deep.

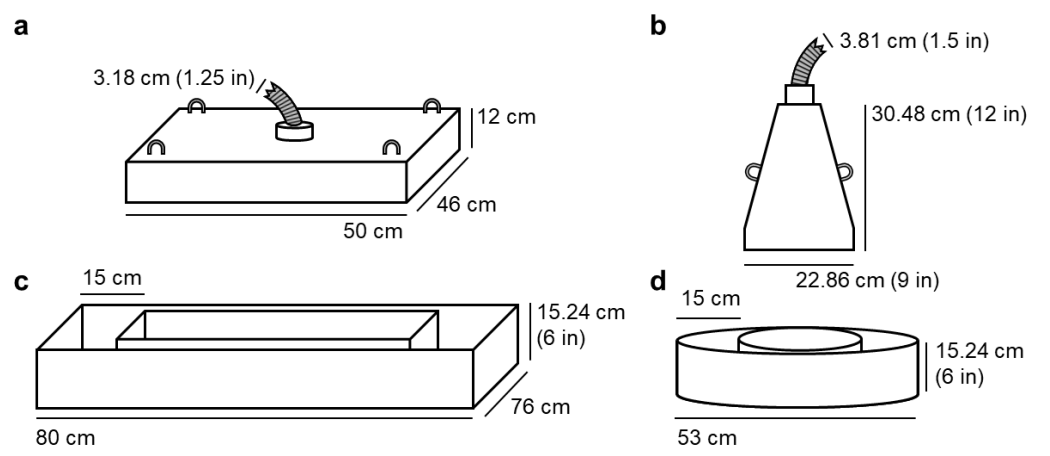


Illustration 2.1. Diagram of the steel 'box' (a) and 'cone' (b) intake nozzles and corresponding plastic barriers (c, d) used to control seeded egg placement on the substrate. Each intake fit snugly inside the corresponding inner barrier. Threaded rods and nuts (not included in diagrams) connected the inner and outer barriers to maintain even spacing.

We also expected the design of the intake nozzle to influence sampling efficiency based on the area over which suction is dispersed onto the substrate. We compared the sampling performance of two contrasting intake designs: a narrow, cone-shaped intake and a wide, box-shaped intake. The ‘box’ intake (Illustration 2.1a) was based on a design used to assess Cisco (*Coregonus artedi*) spawning habitat use with a similar pump setup (George et al. 2017). While the box had a relatively

large sampling area, we suspected that the larger opening likely reduced its sampling efficiency compared to a smaller intake if used with the same pump setup. Additionally, the size and weight of the box design made deployment difficult through winter ice cover (George et al. 2017). Thus, we designed a smaller, round 'cone' intake (Illustration 2.1b) to test if concentrating suction over a smaller area could increase sampling performance, and to enable deployment through a standard 10 in. (25.4 cm) ice auger hole for use on lakes in winter.

This experiment was designed to assess the sampling efficiency of a stationary benthic pump sampler with respect to three key inferential objectives related to the spawning ecology of relevant fishes: (1) detecting egg presence, (2) measuring differences in relative egg abundance, and (3) estimating absolute egg abundance. At the coarsest level of inference, observing the presence or absence of incubating eggs can inform large-scale spawning site identification (e.g. George et al. 2017, Roseman et al. 2002), perhaps in the context of colonization and extirpation. Within a spawning area, relative egg abundance and survival coupled with fine-scale habitat information can inform local habitat selection and habitat suitability models (e.g. Dorr III et al. 1981, Fitzsimons 1995, Roseman et al. 1996). Finally, estimates of the absolute abundance of eggs can be used to infer egg loads and possibly to inform population dynamics models (e.g. Schweigert 1993). Here we present results from replicate field trials and examine the effects of varying substrate type, gear design, sampling effort, and egg density on the ability of the pump sampler to address the above objectives.

Methods

Field trials

We used a gasoline-powered diaphragm pump rated for 66 gal/min (approx. 250 L/min). Ribbed pool hose connected an intake nozzle to the pump intake, which was reduced with additional fittings from 3 inches in diameter to 1.5 or 1.25 inches depending on the hose used for each intake (Illustration 2.1a, b). We tested two intake nozzle designs differentiated by sampling area (Illustration 2.1a, b). Both were designed to sit upright on the substrate while sampling, and were constructed from

steel with exterior eyes for attaching lines to relieve tension from the hose during deployment. The intakes did not include a screen to prevent small rocks from entering and damaging the pump, as diaphragm pumps are less likely to experience internal damage from debris than centrifugal pumps.

Based on prior field trials, we suspected that the pump may collect eggs from an area greater than the opening of the intake nozzle. To test for this, we constructed plastic barriers (Illustration 2.1c, d) to define two areas where eggs were seeded for each trial: the area directly under the intake and a "buffer" area that extended 15 cm out from the perimeter of the intake. Prior observations of the pump excavating silt during sampling suggested that this buffer area included the extent of the potential sampling reach of each intake.

We tested the pump on four different substrate types defined by particle size (according to Wentworth 1922) and depth of rock layers for hard substrates: silt, single layer of gravel, single layer of cobble (shallow), and three layers of cobble (deep). These were either naturally occurring or easily constructed at our study site, a small, shallow reservoir along a large stream in central New York State, where we completed this work in late summer and early fall. We selected an area that was near-shore, 1-2m deep, and was accessible by wading or snorkeling for seeding eggs and running the trials. The reservoir had a base layer of compacted mud covered with thin layers of natural silt and gravel. Shallow and deep cobble substrates were constructed on top of the compact mud layer with rounded cobble collected from the nearby stream bed.

Commercially available Lake Whitefish (*Coregonus clupeaformis*) caviar was used as a proxy for non-adhesive eggs of similar size (mean diameter 2.35 mm SD 0.166 mm) to many freshwater broadcast spawning fish species (e.g. Auer 1982). Eggs were rinsed to remove the brine preservative, resulting in a buoyancy similar to that of natural eggs (mean settling velocity in fresh water of 3.8 cms^{-1}, compared to ~3.3 cms^{-1} for *Coregonus spp.* eggs reported by Barton et al. 2011). The eggs were counted to achieve densities of 50, 200, or 500 eggs m^{-2} ("low," "medium," and "high") for each trial and dyed different colors to identify those seeded in the intake versus buffer sampling areas. Both sampling areas were always seeded with the same density of eggs within a single trial.

The pump was located nearby on shore, nearly at water level to approximate its placement and resulting head pressure if used on a small boat or ice. For each trial, the plastic barriers were placed on clean substrate and eggs were dispersed underwater onto the substrate within the intake and buffer areas. The pump was started and the intake was then lowered into the inner barrier until it touched the substrate—marking the start of the sampling period. The barriers were then immediately lifted, taking care not to disturb the eggs. Sampling continued for two sequential two-minute periods to measure egg collection over time. The pump outflow was directed into one of two sieve-bottom buckets to collect eggs separately from each sampling period. In prior trials most eggs were collected within the first minute or two, so we expected that sampling would be complete by four minutes. At four minutes, the intake was lifted from the substrate but kept underwater to flush the hoses. Material collected in the buckets was rinsed into trays and eggs were picked out and counted. We conducted eight replicate trials for each combination of the two intake designs, four substrate types, and three egg densities.

Data analysis

Data from replicate trials were considered in three forms for analysis: binary presence detection outcomes, egg recovery ratios, and estimates of absolute egg density. Egg presence was correctly detected if at least one egg was recovered from either the intake or buffer sampling area. Egg recovery ratios refer to the total number of recovered eggs, from both the intake and buffer areas, divided by the number of eggs seeded in only the intake area. These ratios were intended to indicate bias from true abundance within the intake area. Absolute density estimates were calculated by dividing the total number of recovered eggs, from both the intake and buffer areas, by the spatial area directly under the intake. Except when noted, analyzed data were from the complete four minutes of pumping.

All analyses were performed using R statistical language (R Core Team 2019). We constructed regression models separately for each intake design for each of the three forms of data described above. All models included substrate type, egg density (categorical), and an interaction between substrate and density as predictors. Logistic regression models were used for the

presence/absence results, yielding estimated probabilities of detecting presence. In some cases, no eggs were collected in any of the replicate trials, presenting issues with 'complete separability' for maximum likelihood-based logistic regression analysis. To address this, we used Bayesian implementation of logistic regression which can provide valid inference in the face of separability (Gelman et al. 2008). Bayesian logistic regression was carried out using bayesglm() in the 'arm' package (Gelman and Su 2018), which defaults to using a weakly informative Cauchy prior distribution for the coefficients as recommended by Gelman et al. (2008). Bayesian regression coefficients and model prediction estimates were reported as posterior means from 10,000 posterior draws (simulated from estimated posterior distributions for fitted parameters; Gelman and Su 2018). For consistency, we also used this Bayesian framework to construct Normal linear regression models for egg recovery ratios and absolute density estimates. Recovery ratio data were square root transformed and density estimates were cube root transformed to satisfy assumptions of normality. For both of these sets of models, prediction estimates were calculated for each posterior draw and median values across draws were back-transformed to the predictor scales.

Results

Sampling area and duration

We routinely observed egg recovery from the buffer area, outside the intake area (Table 2.1). When using the box intake, eggs were recovered from the buffer area in 62% of all trials with any eggs recovered, and in 73% of these trials when using the cone. In some cases—13% of trials when using the box and 35% with the cone—more eggs were collected from the buffer area than from the intake area. Most eggs were recovered during the first two minutes of sampling, however, some additional egg recovery often occurred during the second two minutes (Table 2.1). This was more common when using the box, for which 42% of all recovered eggs, from either sampling area, were collected in the second two minutes of pumping compared to only 14% when using the cone.

Table 2.1. Proportions of replicate trials (n = 8) with any eggs recovered from the buffer area outside the intake (from the total four minutes of sampling), and from the first and second halves of the four minute total sampling period (from both the intake and buffer areas). Density refers to the seeded density of eggs: 50 (Low), 200 (Medium), and 500 (High) eggs m^{-2}.

		Proportion of replicate trials with any eggs recovered from:					
		Buffer area		0-2 min. period		2-4 min. period	
Substrate	Density	Box	Cone	Box	Cone	Box	Cone
Silt	Low	0.625	0.5	0.875	0.875	0.5	0.125
	Med.	0.875	0.875	1	1	1	0.625
	High	1	1	1	1	1	0.75
Gravel	Low	0.25	0.125	0.25	0.5	0.25	0.125
	Med.	0.125	0.875	0.25	1	0.375	0.75
	High	0.5	0.75	0.5	1	0.875	0.375
Cobble	Low	0	0.5	0.125	0.875	0	0.125
(shallow)	Med.	0.125	0.75	0	1	0.125	0.375
	High	0	1	0.25	1	0.125	0.75
Cobble	Low	0	0.125	0.125	0.25	0	0
(deep)	Med.	0	0	0.125	0	0	0
	High	0	0	0	0.125	0	0.125

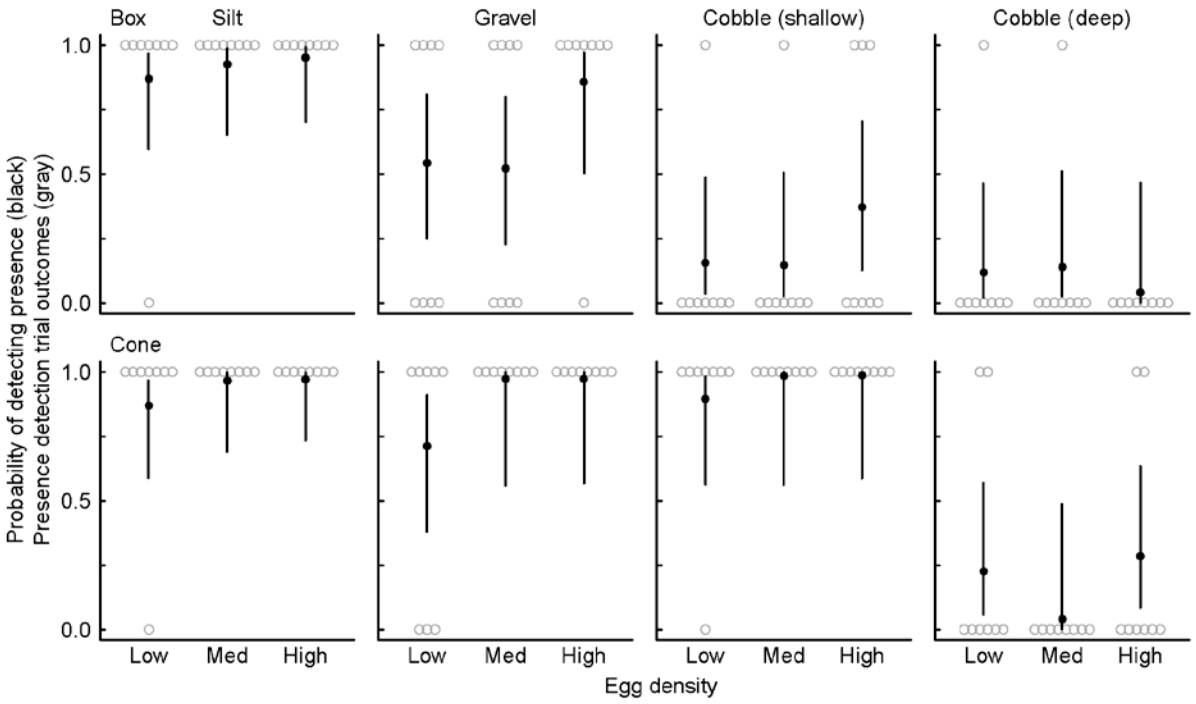


Figure 2.1. Egg presence detection outcomes from replicate trials (n = 8, open gray circles) and posterior estimates of presence/absence detection probability (black circles) with 95% credible intervals (black lines) from Bayesian logistic regression models for the box intake (top row) and cone intake (bottom row). Empirical egg presence results take on a value of either one, indicating at least one egg was collected, or zero, indicating no eggs were collected. Egg density refers to the seeded density of eggs: 50 (Low), 200 (Med), and 500 (High) eggs m^{-2}.

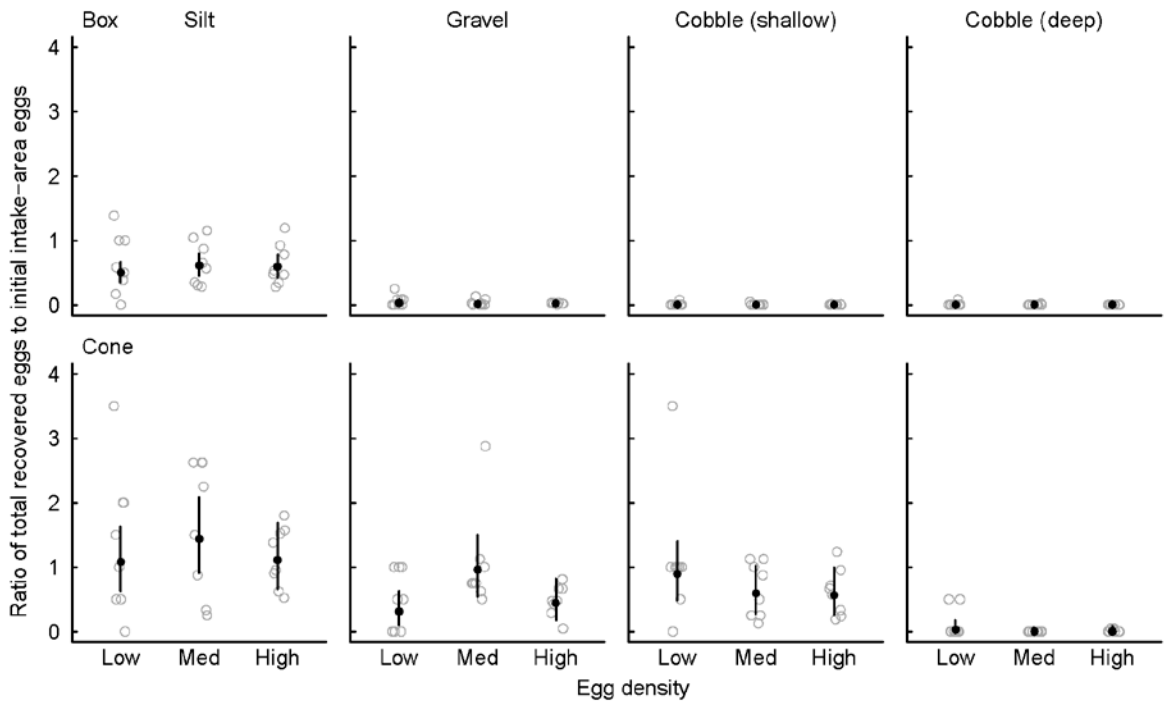


Figure 2.2. Egg recovery ratios from replicate benthic pump sampler trials (n = 8, open gray circles) and posterior estimates of egg recovery ratios (black circles) with 95% credible intervals (black lines) from Bayesian Normal linear regression models for the box intake (top row) and cone intake (bottom row). Egg density refers to the seeded density of eggs: 50 (Low), 200 (Med), and 500 (High) eggs m^{-2}.

Presence/absence detection

On silt substrate, both intake designs detected eggs in almost all trials, resulting in high mean regression estimates of egg presence detection probabilities across all egg densities on this bottom type (box: 0.93, cone: 0.92; Figure 2.1). Using the box, we observed decreasing performance on gravel (0.64) and shallow cobble (0.22) across all egg densities. In contrast, the cone intake maintained high detection probabilities across densities on gravel (0.89) and shallow cobble (0.96). Both intake designs were mostly ineffective at detecting presence on deep cobble (box: 0.10, cone: 0.18).

Egg recovery ratios

Egg recovery ratios, the total number of eggs recovered (from both seeded areas) divided by the initial number of eggs seeded in the intake area, were highly variable across replicate trials within gear-substrate-density combinations (Figure 2.2). Posterior 95% credible intervals were generally

overlapping across densities on silt with the box, and across densities and substrates—excluding deep cobble—with the cone. For the box intake, egg recovery ratios from individual trials only exceeded one in a few trials on silt. Mean posterior estimates of recovery ratios for the box across all densities were 0.57 on silt, 0.02 on gravel, and 0.001 on both cobble substrates. For the cone, mean posterior estimates of recovery ratios for each egg density were all above one on silt (low: 1.08, medium: 1.44, high: 1.11), but less than one across densities on gravel (0.57), shallow cobble (0.68), and deep cobble (0.01).

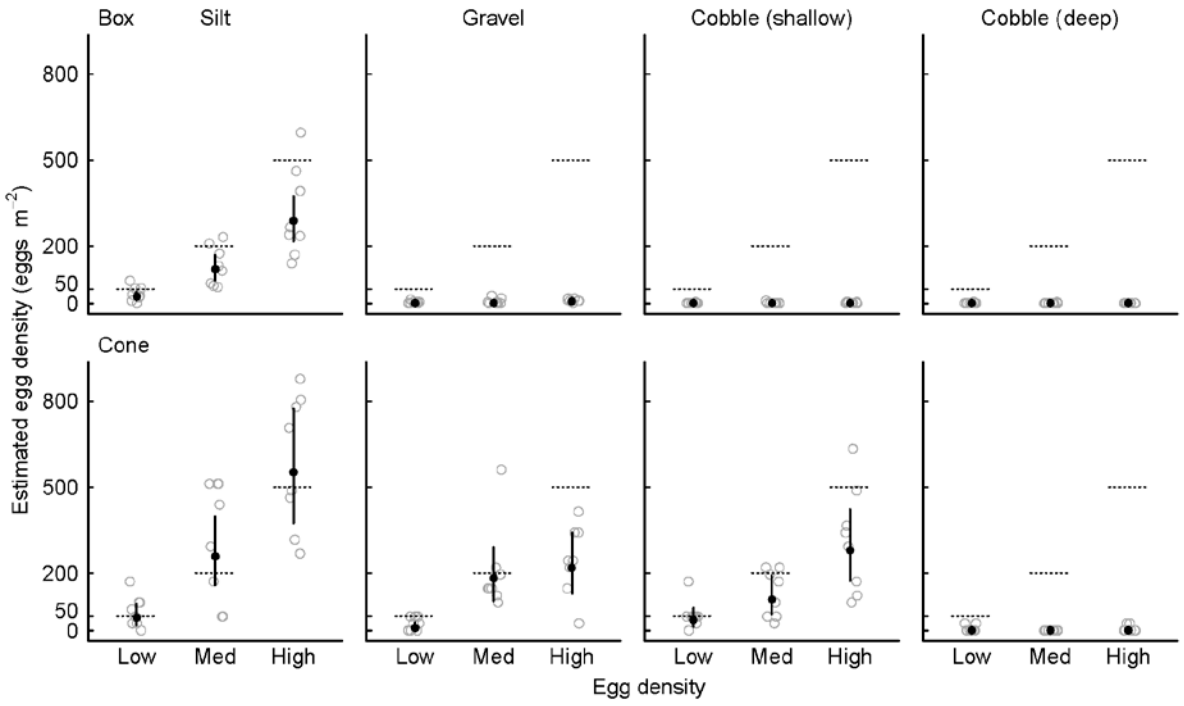


Figure 2.3. Absolute egg density estimates from replicate benthic pump sampler trials (n = 8, open gray circles) and posterior estimates of egg recovery proportions (black circles) with 95% credible intervals (black lines) from Bayesian Normal linear regression models (Table A.3) for the box intake (top row) and cone intake (bottom row). Egg density refers to the seeded density of eggs, with true numeric values indicated by the horizontal dashed lines: 50 (Low), 200 (Med), and 500 (High) eggs m^{-2}.

Absolute egg density estimates

Absolute egg density estimates generally reflected the correct ordinal ranking with respect to true seeded densities when using the box on silt, and the cone on silt, gravel, and shallow cobble (Figure 2.3). The box intake, however, failed to achieve reasonable egg density estimates for any seeding density on all three rocky substrates, and credible intervals for model estimates did not include the true densities. In contrast, the cone intake was able to achieve mean egg density estimates consistent with true seeded densities for these bottom types for low to medium seeding densities. On silt, the cone density estimates were also consistent with the true density, however, results diverged somewhat for the high egg densities on gravel and shallow cobble. On silt, the cone recovered enough eggs from the buffer area to result in a slight positive bias in median density estimates for all densities on silt, whereas median density estimates were negatively biased across densities on gravel and shallow cobble. As with presence/absence and egg recovery proportion performance, both intake types failed to sample eggs on the deep cobble substrate.

Discussion

Stationary benthic pumps can be effective at sampling eggs across a range of substrates from fine silt up to shallow layers of larger rocky particles. Additionally, our observations of variable pump performance by intake design and substrate type emphasizes the importance of assessing sampling effectiveness for novel gears prior to their deployment for ecological study. Overall, the cone intake design was considerably more effective than the box intake in detecting the presence of eggs and in providing relative and absolute measures of egg density across a range of substrates. Although the box intake may have merits because of its ability to sample a relatively larger substrate area, we found that its usefulness is restricted to sampling on fine-particle substrates.

Observations of egg presence provide useful information for understanding spawning ecology and informing management, including monitoring for population recovery and surveillance for unknown spawning grounds (e.g. Roseman et al. 2007, George et al. 2017). In this regard, the cone

intake design had high and consistent ability to detect egg presence across a range of substrates from silt up to multiple layers of cobble, and thus may be a reliable tool for detecting presence of benthic eggs across sites. Additionally, the comparative ease of deployment for the cone intake versus the box could facilitate more efficient egg presence surveys across larger spatial scales, important for assessing the spatial extent of spawning areas.

The pump sampler and cone intake may also be an acceptable gear for developing indices of relative egg abundance—useful for assessing spawning habitat preference within known spawning grounds or for monitoring egg losses over time, among other applications (e.g. Bouwes and Luecke 1997, Dorr III et al. 1981). We note, however, that at the highest egg densities we tested, the cone intake exhibited lower egg recovery and downward-biased density estimates on gravel and shallow cobble. Since we didn't observe this on silt, this pattern could be a result of the intake displacing some eggs through interstices in the substrate below the plastic barriers during deployment. Thus, we caution that additional testing may be warranted to assess egg recovery abilities of benthic pumps in cases where high egg loads are expected (e.g. Lake Trout (*Salvelinus namaycush*) have been observed to produce >6000 eggs m^2, Fitzsimons, 1995).

Finally, estimating absolute egg abundance places the greatest demand on the robustness of pump sampling data, requiring that egg recovery is high and consistent to produce unbiased density estimates. The box intake only provided reasonable estimates of density on silt, and these were negatively biased. The cone intake produced more accurate density estimates across substrates, but with varying directions of bias and variability across replicate trials that obscured separation between densities. We measured both positive (on silt) and negative (on gravel and shallow cobble) bias—a result of differential sampling reach and penetration into interstitial space dependent on the type of substrate. Observations of positive bias in density estimates on silt (frequently when using the cone, and in a few instances when using the box) indicated frequent retrieval of eggs from outside the area directly under the intake, particularly on soft sediment where the intake sometimes 'excavated' a pit

into the sediment. Thus, the best intake design for measuring absolute egg abundance likely depends on substrate type being sampled.

Alterations to the pump systems and intake designs may improve the effectiveness of this sampling method. The higher efficiency of the narrower cone intake over the wider box intake shows that increased suction does improve egg collection. We suspect that an intake with a larger sampling area could be more effective if used with a pump with a higher flow rate, but higher flow may also expand the potential sampling radius resulting in overestimation. When sampling in deep water, a longer intake hose could negatively affect sampling efficiency if the cumulative force of friction between water and the inside of the hose is strong enough to reduce suction at the intake. A longer intake hose also has a higher risk of collapsing due this type of head pressure, especially if using a pump with a relatively high flow rate.

While mechanical improvements or additional features, such as a hose directed under the intake to flush the substrate, may increase sampling performance, it is likely not feasible to design a pump sampler that can effectively sample the deepest interstitial spaces. This leaves a need for alternative sampling methods for deep rocky habitats, such as mesh bags buried by divers (e.g. Perkins and Krueger 1984).

Even with the limitations outlined above, the pump sampler is a useful tool for obtaining information about benthic spawning fish ecology. Benefits of the stationary pump sampler include high-resolution information about egg distribution, the ability to observe incubating eggs in situ, relatively low cost, high portability, durability under adverse conditions, and ease of deployment.

CHAPTER 3

DIFFERENTIATION BETWEEN LAKE WHITEFISH AND CISCO EGGS BASED ON DIAMETER

Abstract

Cisco (*Coregonus artedi*) and Lake Whitefish (*Coregonus clupeaformis*) are native fish species of management concern in the Great Lakes. Both often overlap in spawning locations and timing, therefore, species-level inference from in situ samples of eggs is not possible if eggs cannot be differentiated. Genetic barcoding and rearing of live eggs until hatched larvae can be visually identified can distinguish between the species, but can be time or cost intensive. Sparse evidence in the literature suggests that Lake Whitefish eggs are consistently larger than those of Cisco in the Great Lakes, but this has not yet been substantiated. Samples from Cisco and Lake Whitefish spawning grounds are unlikely to contain similar eggs of other species, thus, we compiled sets of egg diameters of known identity to assess whether Lake Whitefish and Cisco eggs could be differentiated based on size alone. Overall, mean Lake Whitefish egg diameter (3.06 mm, SD = 0.17, n = 430) was larger than for Cisco eggs (2.41 mm, SD = 0.17, n = 1203). Egg size and variance differed minimally between two source locations and between hatchery and in situ sourced eggs, possibly reflecting other sources of variability such as hatchery processing and maternal condition. We used the standard classifier CART with a bootstrapping approach to identify a size classification threshold of 2.85 mm (95% CI: 2.83, 2.86). Discrimination of Lake Whitefish and Cisco eggs by diameter is potentially accurate, time- and cost-efficient, and may be especially suitable for large sample sizes.

Introduction

Survival of early life stages of fish can influence recruitment rates and population demographics, making the study of early life history potentially important for informing their biology and management (Houde 1997). One approach to collecting this information is sampling eggs in situ

from spawning areas, a method currently used to study Cisco (*Coregonus artedi*) and Lake Whitefish (*Coregonus clupeaformis*), native species of management concern in the Great Lakes (George et al. 2017, Roseman et al. 2007, Stewart et al. 2017). However, both spawn in late fall, often in the same locations, and their non-adhesive, visually similar eggs incubate on the substrate through winter (Behmer et al. 1980, Pritchard 1931). Samples from the field could potentially include eggs from either or both species, thus making species-level inferences from potentially mixed samples of eggs requires the ability to differentiate Cisco and Lake Whitefish eggs.

Importantly, egg samples collected from these locations between late fall and early spring are unlikely to include visually similar eggs from other species. Lake trout (*Salvelinus namaycush*) eggs may be present, but are relatively large, pigmented, and thus are easily distinguished from Cisco or Lake Whitefish eggs (Auer 1982). Other predatory salmonids also have larger, pigmented eggs compared to Cisco and Lake Whitefish, and many spawn in tributaries rather than nearshore areas, or at a different time of year (Auer 1982). Bloater (*Coregonus hoyi*) eggs are similar in appearance, however, they spawn in areas deeper than those where Cisco and Lake Whitefish spawn together, and often spawn at a different time of year (Auer 1982, Scott 1973).

Previous studies have used genetic techniques such as DNA barcoding (e.g. George et al. 2017), or rearing of live eggs in hatcheries and visually identifying the larvae (e.g. Roseman et al. 2007) to differentiate Cisco and Lake Whitefish eggs. These methods can be relatively costly and time intensive, and visual identification of larvae may be unreliable for some populations (George et al. 2018, Hulley et al. 2018). Literature records and anecdotal observations suggest that in the Great Lakes, Cisco eggs are smaller on average than eggs of Lake Whitefish (e.g. Hinrichs 1979). However, we found that published reports of egg sizes are limited in number, lack accompanying variance and methods, and do not allow comparison among populations of these species from the same locations (Table 3.1). Therefore, existing evidence is insufficient to substantiate the use of egg size as a viable metric for differentiating eggs of these two species. Here, we compare size estimates for fertilized

Cisco and Lake Whitefish eggs and identify a single size classification threshold using data sets from two locations in the Great Lakes, collected from hatchery stock and in situ field samples.

Table 3.1. Published egg diameter estimates for Cisco and Lake Whitefish in the Great Lakes region. Dashes indicate information that was not reported. All measurements were from eggs artificially fertilized and incubated in controlled conditions after collecting spawning adult fish using gillnets, trap nets, or seine nets.

				Adults		Eggs					
			Spawn	n	Length	Hours post-			Diameter (mm)		
Species	Source	Location	year	(f/m)	(mm)	fertilization	Preserved	Measurement	Mean	SE/SD	n
Lake Whitefish	a	Lake Superior	1966	2/2	(std.) 255-422	4	no	von Bayer	2.95	0.01 / -	100
	b	Door Co., WI, L. Mich.	1975	-	-	48	formalin	microscope	2.9	- / 0.20	41
	c	Fishing Is., L. Huron	2014	3/6	-	24	no	microscope	3.07	- / 0.19	18
	d	Fishing Is., L. Huron	2013	18/30	(fork) ~500	8	no	microscope	3.19	- / 0.16	-
Cisco	a	Lake Superior	1966	2/2	(std.) 229-365	4	no	von Bayer	2.1	0.01 / -	100
	b_1	Sunset Lake, WI	1976	-	-	48	formalin	microscope	2.0	- / 0.03	39
	b_2	Pallette Lake, WI	1975	-	-	24-72	formalin	microscope	2.0	- / 0.03	29

a Booke, H. E. 1970
b Hinrichs, M. A. 1979
c Lim, M. Y.-T., et al. 2017
d Sreetharan, S., et al. 2015

Methods

Data sources

Individual egg measurements were assembled from multiple sources (Table 3.2), including hatchery-sourced eggs and field samples. Eggs were collected via both sources from two locations in the Great Lakes (Chaumont Bay, Lake Ontario and Elk Rapids, Grand Traverse Bay, Lake Michigan). Hatchery-sourced eggs were stripped from ripe females and fertilized by mixing with milt stripped from males. Fertilized eggs were kept separately by species and incubated for at least four hours post-fertilization to ensure they were water hardened (e.g. Brooke and Colby 1980). Eggs were then photographed under magnification while alive or after preservation in 95% ethanol. Field-sourced eggs were collected using egg mats anchored to the substrate to trap settling eggs during spawning. These were constructed using rectangular steel frames (approx. 40 by 20 cm) wrapped with hogs hair furnace filter mats secured to the frames with binder clips (e.g. Roseman et al. 2011). Eggs were picked from the mats and preserved in 95% ethanol before being photographed under magnification, then were placed individually in vials in preparation for genetic species identification.

Table 3.2. Summary of fertilized egg diameters for Cisco and Lake Whitefish from data sets used to calculate a species classification size threshold. Sampling method indicates if eggs were collected from the field using egg mats, or were stripped from spawning adults for hatchery rearing.

		Spawn	Sampling	Days post-		Cisco				Lake Whitefish			
Source	Location	year	method	fertilization	Preserved	Mean	SE	SD	n	Mean	SE	SD	n
1[a]	Chaumont Bay, L. Ont.	2017	egg mats	1-14	ethanol	2.50	0.022	0.28	154	3.25	0.032	0.16	26
1[b]		2018	egg mats	1-14	ethanol	2.46	0.018	0.22	149	3.20	0.034	0.24	49
2		2018	hatchery	10	ethanol	2.38	0.004	0.06	294	2.96	0.003	0.05	202
3	Elk Rapids, L. Mich.	2016	hatchery	11-13	no	2.40	0.007	0.16	493	3.10	0.016	0.18	129
4		2018	egg mats	1-14	ethanol	2.39	0.012	0.13	113	3.19	0.028	0.14	24
					Total	2.41	0.005	0.17	1203	3.06	0.008	0.17	430

1 This study
2 US Geological Survey, Tunison Laboratory
3 Little Traverse Bay Bands of Odawa Indians Natural Resources Department
4 Michigan Department of Natural Resources and The Nature Conservancy

Fiji, a distribution of ImageJ software, was used to measure all eggs (Schindelin et al. 2012). Scale in millimeters was set for each image using length references within the images. The ellipse tool was used to obtain a mean diameter for each egg, with precision to the hundredths place. Only eggs completely within the frame and not damaged or irregular in shape (i.e. lysed or shriveled) were measured. A subset of eggs from egg mat samples were measured before and after preservation in ethanol and experienced a <1% decrease in size from preservation, thus, data from unpreserved eggs were retained.

Eggs collected from the field were identified to species via barcoding of the mitochondrial cytochrome c oxidase I (COI) gene (George et al. 2017). DNA was extracted from eggs using a DNEasy Blood & Tissue Kit (Quiagen, Valencia, CA). PCR was used to amplify copies of the COI gene using primers VR1_t1 and VF2_t1 (Ivanova et al. 2007), which were then cut at specific sequences with the restriction enzyme Eco109I (George et al. 2017). DNA fragment sizes were visualized with gel electrophoresis, and resulting banding patterns were matched with control samples for each species which were included in the gels.

Data analysis

The standard classifier CART (classification and regression tree) was implemented in R statistical language (R Core Team 2019) and used to identify a size classification threshold based on all pooled egg measurements (Table 3.2). This approach iteratively splits data into binary groups by identifying a predictor value from the data that minimizes a measure of misclassification of the response variable within groups. We used the rpart function in the rpart package (Therneau and Atkinson 2018), with ‘method’ set to ‘class’ and all other arguments set to the default values. Classification tree models were fit with egg diameter, location (Chaumont Bay or Elk Rapids), and collection method (hatchery or field) as predictors of species to account for possible effects of sample source. No interactions are specified for CART models, as important interactions are reflected in the tree structure. Bootstrapping was used to calculate a mean size threshold and 95% confidence interval by fitting the model to a randomly selected subset of the data (~80% of observations) over 1000

iterations. We used a confusion matrix to compare accuracy and error rates for each species when classifying all pooled data using the size threshold.

Results

Overall mean egg diameters were 2.41 mm (SE = 0.17, range: 1.88-3.12, n = 1203) for Cisco and 3.06 (SD = 0.17, range: 2.59-3.78, n = 430) for Lake Whitefish (Figure 3.1a). Egg sizes were generally similar within species across samples, though hatchery eggs from Chaumont Bay were notably less variable and slightly smaller than other samples, especially Lake Whitefish eggs. A single size classification threshold of 2.85 mm (95% confidence interval: 2.83, 2.86) was identified using pooled egg measurements. When used to classify all data, accuracy rates were 97.8% overall, 98% for Cisco, and 96% for Lake Whitefish (Table 3.3). Literature values were broadly consistent with the size threshold, but were generally smaller than eggs in our data sets (Table 3.2, Figure 3.1b).

To better understand the impact of observed variability in size distributions between samples, especially considering the smaller size and variance of Lake Whitefish eggs from Chaumont Bay hatchery stock relative to other sources (Figure 3.1a), we adjusted the complexity parameter (default value 0.01) to 0.009 to allow additional splits that previously did not sufficiently increase classification performance. In this case, location was still not included, and collection method was only used in a terminal split. The relatively small effect of collection method was reflected in its low variable importance score (2, versus 98 for diameter) calculated by the rpart function. In addition, we calculated the size threshold again excluding the hatchery data and found that the size threshold was only slightly larger: 2.89 mm (95% confidence interval: 2.88, 2.98) and had similarly high accuracy rates when applied to the total pooled data (97.2% overall, 98% for Cisco, and 92% for Lake Whitefish; Table 3).

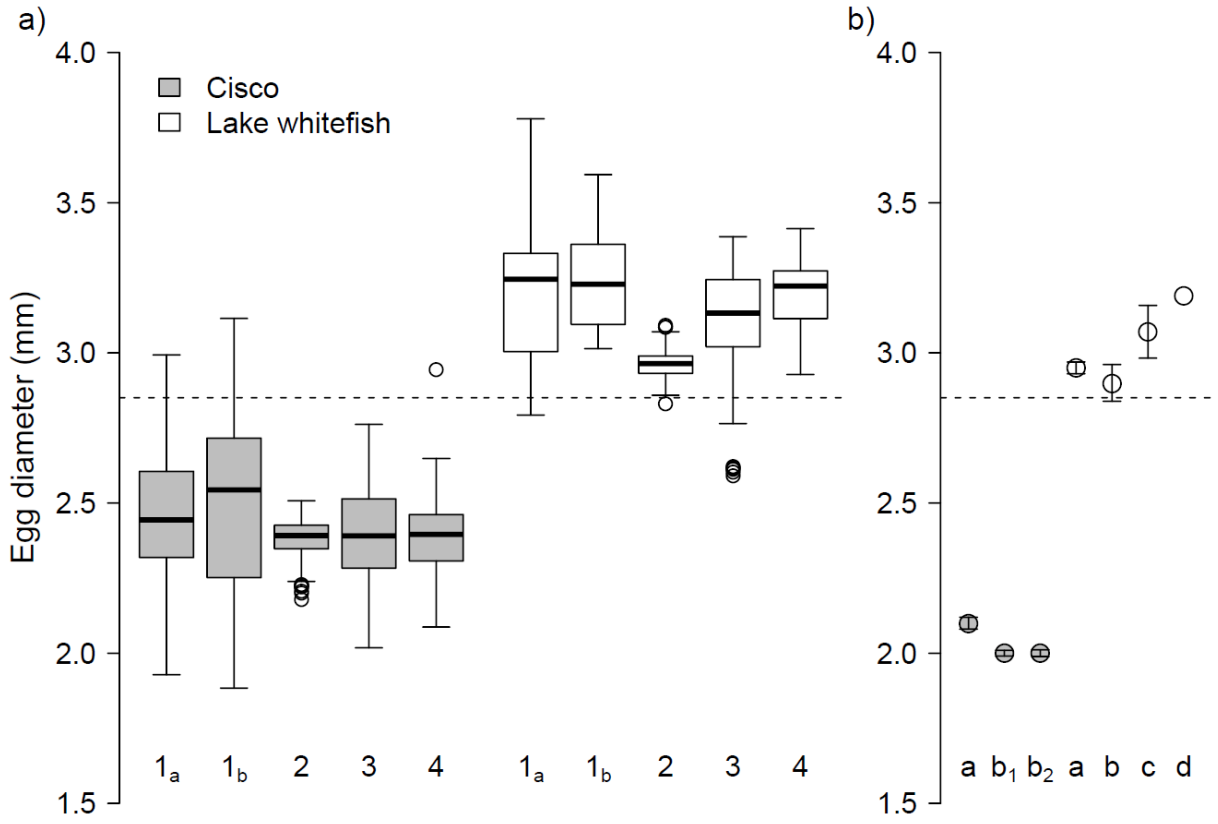


Figure 3.1. Distribution of Cisco (grey) and Lake Whitefish (white) egg diameters (a) in samples (numbers refer to samples in Table 1) used to calculate a size classification threshold (2.85 mm, dashed line in both panels), and (b) egg diameter estimates ± 1.96(SE) from published literature (letters refer to Table 2).

Table 3.3. Confusion matrices comparing counts of correct (along the diagonal) and incorrect (adjacent to diagonal) predicted species assignments ("Prediction") for egg diameters of known identity ("Reference"). Species assignments were determined using size thresholds derived from all data sets (a) and from only field-collected data (b).

a)

	Reference	
Prediction	Cisco	Lake Whitefish
Cisco	1184	17
Lake whitefish	19	413

b)

	Reference	
Prediction	Cisco	Lake Whitefish
Cisco	1190	33
Lake whitefish	13	397

Discussion

Across all of our data sources, we found that Lake Whitefish eggs were consistently larger than Cisco eggs, allowing us to calculate a size classification threshold that could be used to differentiate eggs of each species with relatively high accuracy. Some variation in sizes between eggs collected from hatchery stock and those collected from the field were observed, but were not important relative to species-based differences. We suspect that the low variance and slightly smaller size of hatchery sourced eggs from Chaumont Bay could reflect maternal effects on egg size, as all eggs for both species were stripped from a single female of each species (Wang et al. 2012). Hatchery-sourced eggs from Elk Rapids, in contrast, were derived from a mix of multiple females and were more consistent in size and variance with eggs collected from the field for both species. Intraspecific egg size variation in the natural environment is likely a result of multiple influences, including ecosystem productivity, temperature regimes, stock-specific traits, and individual maternal effects (Feiner et al. 2016, Morrongiello et al. 2012). This could also explain some of the differences between our data and values from the literature, which were derived from other populations in the Great Lakes and from smaller inland lakes.

Differences in egg sizes between Cisco and Lake Whitefish were relatively large among our data sets, consistent with the limited available literature and the hypothesis that differential responses to selection pressures may have driven this difference (Booke 1970). The larger eggs of Lake Whitefish represent a greater investment in energy per egg, but likely result in larger larvae with higher survival rates, whereas the smaller eggs of Cisco represent a strategy of investing less energy per egg allowing for higher egg production (Booke 1970). These alternate strategies could reflect mechanisms of speciation and intraspecific population segregation in Cisco and Lake Whitefish, and more broadly in the genus *Coregonus* (Todd et al. 1981).

Using egg size in early life history studies to differentiate potentially mixed samples of Cisco and Lake Whitefish eggs thus warrants consideration of population-level sources of variation. We suggest that validation of the classification threshold should be conducted for populations not

represented in this study. We reported a threshold to the hundredths of a millimeter with little variance, but the optimal splitting size and associated accuracy rates could vary depending on the populations being studied.

In addition, automated image analysis could support size-based classification if used to measure egg sizes from images. For a subset of our data, we wrote an R script using the package EBImage (Pau et al. 2010) that automatically detects and measures eggs within images of magnified eggs. This approach was ultimately not used in our study as the image processing steps are tuned to a specific set of image attributes, such as contrast and lighting direction (e.g. from below or above), which varied among image sets. However, the simplicity of size-based differentiation could be valuable for large sample sizes relative to other species identification techniques, especially if using consistent imaging conditions and an automated measurement approach. In addition, size-based differentiation could be used as a preliminary assessment of the presence of Cisco or Lake Whitefish in egg samples to determine if species verification via other methods is warranted.

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